## II. REMARKS

**Formal Matters**

Claims 36-50 are pending after entry of the amendments set forth herein.

Claims 36-49 were examined. Claims 36-49 were rejected.

Claim 50 is new. Support for new claim 50 is found in the claims as originally filed, specifically in claim 44, and in Figure 1.

Accordingly, no new matter is added by these amendments.

**Rejections Withdrawn**

Applicants gratefully acknowledge withdrawal of rejections of claims under 35 U.S.C.§103(a), as detailed in sections 6-8 in the Office Action dated July 18, 2002.

**Interview**

Applicants wish to extend their gratitude to Examiner Helms for the interview on October 15, 2002, with applicants' representatives James Keddie and Carol Francis. The outstanding rejections under 35 U.S.C. §103(a) were discussed during the interview, as well as arguments to overcome the rejections.

**Rejections under 35 U.S.C. §103**

*Summary of the outstanding rejections*

Claims 36-40 and 42-49 have been rejected under 35 U.S.C. §103(a) as obvious over Horwitz *et al* (PNAS 85:8678-8682) in view of Cregg *et al* (Developments in Industrial Microbiology 29:33-41, 1998), The Invitrogen 1997 Catalog, Sambrook *et al* (Molecular Cloning: A Laboratory Manual, Second Edition, 1989), and Robinson *et al* (US Patent 6,204,023).

Claim 41 has been rejected under 35 U.S.C. §103(a) as obvious over Horwitz *et al* (PNAS 85:8678-8682) in view of the secondary references cited above, in further view of Vanderlaan *et al* (US Patent 5,429,925).

These rejections are respectfully traversed.

*The Law of Obviousness*

It is well known that in order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.[1,2] Second, there must be a reasonable expectation of success.[3] Finally, the prior art reference, or references when combined, must teach or suggest all the claim limitations.[4] All three criteria must be met.

Furthermore, as recited in the MPEP at §2141.02 "A prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention." [emphasis in the original].

As will be demonstrated below, no reference or combination of references cited by the Office reasonably provides motivation to combine the references to obtain the claimed invention. In fact, when the references, particularly Robinson, are each read as a whole, one of skill in the art would be led away from *Pichia* expression vectors that have dual expression cassettes for expression of antibody heavy and light chains, and the use of such vectors for antibody production in *Pichia*, as per the claimed invention.

*a) The cited references provide no motivation to make and use dual expression cassettes for antibody production in Pichia*

Claims 36-40 and 42-49 are rejected for the asserted reason that Horwitz discloses a vector system and method for production of functional antibodies in *S. cerevisiae*, which, when combined with Cregg's *Pichia* alcohol oxidase promoter, Invitrogen's *Pichia* vector system, the general cloning methodologies of Sambrook, and the dual expression cassette vectors of Robinson, renders the claims obvious to one of skill in the art

Claim 41 is rejected for the asserted reason that Horwitz discloses a vector system and method for production of functional antibodies in *S. cerevisiae*, which, when combined with Cregg's *Pichia* alcohol oxidase promoter, Invitrogen's *Pichia* vector system, the general cloning methodologies of

[1] *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988)
[2] *In re Jones*, 21 USPQ2d 1941 (Fed. Cir. 1992).
[3] *In re Merck & Co.*, Inc., 231 USPQ 375 (Fed. Cir. 1986)

Sambrook, the methodologies for producing antibodies in yeast of Robinson, and the anti-dioxin antibody of Vanderlaan renders, the claim obvious to one of skill in the art.

Applicants respectfully traverse each of these rejections.

As discussed during the interview, Robinson is cited on the grounds that it is asserted to provide a key element lacking in each of the other references -- namely a dual expression cassette vector for antibody production in yeast.

However, in the context of yeast, Robinson only discloses single expression cassette vectors, and the only working examples using yeast in antibody production use single expression cassette vectors (Examples 5 and 6).

Furthermore, when read as a whole, the Robinson reference leads one of skill in the art towards using single expression cassette vectors for antibody expression in yeast in several places.

For example:

*i) In column 15, lines 28-38, Robinson discusses his preferred embodiments:*

> "Expression vehicles include plasmids or other vectors. Preferred among these are vehicles carrying a functionally complete human constant heavy **or** light chain sequence having appropriate restriction sites engineered so that any variable heavy **or** light chain sequence with the appropriate cohesive ends can be easily inserted thereinto. Human constant heavy **or** light chain sequence-containing vehicles are thus an important embodiment of the invention. These vehicles can be used as intermediates for the expression of any desired complete heavy **or** light chain in any appropriate host." [emphasis added].

Robinson has indicated that his preferred embodiment is an expression vector that has heavy **OR** light chain encoding sequences, not an expression vector that has that has heavy **AND** light chain encoding sequences. By indicating his preferred embodiment is a single cassette expression vector, Robinson leads one of skill in the art away from use of dual expression cassette vectors as required in the claimed invention.

---

[4] *In re Royka*, 180 USPQ 580 (CCPA 1974)

*ii) Furthermore, in column 45, lines 53-55, Robinson discusses approaches for producing antibodies in yeast:*

> In order for **yeast** to produce an intact functional antibody molecule, a balanced synthesis of both light and heavy chain protein within the host cell is preferred. One approach is to place the light and heavy chain genes **on separate expression vectors** each containing a different selective marker. [emphasis added].

Robinson has disclosed that in order to produce an intact functional antibody in yeast, separate expression vectors should be used, his preferred embodiment. By pointing towards the idea that two single expression cassette expression vectors should be used to produce functional antibodies, Robinson leads one of skill in the art away from the invention.

*iii) Finally, all working examples of antibody production from yeast cells (i.e. Examples 5 and 6) involve single expression cassette vectors.*

Robinson only discloses a dual expression cassette vector and its use for antibody expression in mammalian cells (i.e. Sp2/0 mouse myeloma cell fusion partner cells). The disclosure undeniably indicates that, in the context of yeast, single expression cassette vectors should be used.

*iii) The disclosure of "yeast" in Robinson can not be extended to encompass Pichia*

Robinson uses the terms, "yeast" and "*S. cerevisiae*" interchangeably. For example, Robinson refers to the *S. cerevisiae* gene as "the yeast invertase gene", he refers to the *S. cerevisiae,* the PGK promoter as "the yeast PGK promoter", and refers to the origin of replication of the 2-micron plasmid endogenous to *S. cerevisiae as* "the yeast origin of replication, oriY, a cis-acting sequence (REP3) from the yeast endogenous 2-micron plasmid." At no point in the disclosure does Robinson define "yeast" as anything other than *S. cerevisiae*. Furthermore, the examples provided are examples of antibody expression in *S. cerevisiae*. As such, one of skill in the art would recognize that "One preferred host is yeast" (column 15, line 39) is directed *S. cerevisiae*. *S. cerevisiae* and *Pichia* are very different species, and are not closely related phylogenetically.

Robinson thus fails to teach or suggest that "yeast" means anything other than "*S. cerevisiae*", and does not teach or suggest use of *Pichia*. As such, one of skill in the art, upon reading Robinson, would find no motivation to express an antibody in *Pichia*.

Even if one *were* to interpret the word "yeast" broadly to encompass the species *Pichia*, one of skill in the art would still not be motivated to use a dual expression cassette vector for antibody expression in *Pichia,* since, as noted above, Robinson repeatedly teaches that single expression cassette vectors should be used to express an antibody in yeast, thus leading away from using dual expression cassette vectors in yeast.

As such, based on the Robinson disclosure as a whole, one of skill in the art wishing to express an antibody in yeast would be led away from using a dual expression cassette vector. In short, given the teaching in Robinson that, when yeast is a host cell one should use single expression cassette vectors, there is no motivation to combine the references to provide the claimed invention. Combination of Robinson with the cited references of Horwitz, Cregg, The Invitrogen Catalog or Sambrook does not provide the claimed invention. As such, the criteria required to establish obviousness has not been met, and, accordingly, the rejection of claims 36-40 and 42-49 under 35 U.S.C. §103(a) may be withdrawn.

As for the §103(a) rejection of claim 41, this rejection further relies on a combination of the cited references and Vanderlaan also fails to support a finding of obviousness. Vanderlaan only discloses anti-dioxin antibodies, and does nothing to cure the basic element lacking in the §103 rejection -- i.e., motivation to combine the cited references to provide for the claimed dual expression cassette vector encoding an antibody heavy and light chain, and its use in antibody production in *Pichia.* Therefore, the rejection of claim 41 under 35 U.S.C. §103(a) may also be withdrawn.

*b) One of skill in the art would not practice the invention with any expectation of success*

With respect to claims that require dual expression cassette vectors in which the expression cassettes contain promoters that are related by sequences (e.g. claim 44 and 50), one of skill in the art would not have any reasonable expectation of success in expressing two subunits of an antibody using one vector because of the problems associated with intra-molecular recombination (*e.g.* occurring when two parts of a vector are related), transcriptional interference (*e.g.* occurring when transcription of one cassette reads through an interferes with the transcription of the second cassette), and translational

interference (*e.g.* occurring when transcriptional read-through of one cassette produces an antisense molecule that interferes with the translation of the RNA from the second cassette). Such problems are commonly associated with such dual expression cassette vectors, especially when the expression cassettes contain polynucleotides with similar or identical sequences (for example similar promoters, signal sequence-encoding polynucleotides or terminators).

Examples (abstracts) that relate to these problems, specifically recombination, are provided.

Hoshizaki (Mol Cell Bio, 1985 5:3323-9) shows rearrangement of a plasmid containing the a Drosophila transposable element 412 in *Saccharomyces cerevisiae*. The rearrangement is "most likely is the result of homologous recombination within the long terminal repeats.".

Peterson (J. Bact., 1983 156: 177-85) shows a plasmid that undergoes a rearrangement caused by homologous sequences present flanking a resistance gene in bacteria.

Nies (J. Antimicrob Chemother. 1986 18:Suppl 35-41) shows that multiple copies of IS-elements can cause plasmid rearrangements in bacteria.

In addition, a page of technical material found at Stratagene's website (http://www.stratagene.com/displayProduct.asp?productId=290) states that "Accurately replicating eukaryotic DNA in prokaryotic cells can be problematic. Particular eukaryotic genes may contain inverted repeats or secondary structures....". In the case at hand, the dual expression cassette vectors should have significant secondary structure since each of the cassettes shares considerable sequence identity, and, as such, the production of such a vector would be viewed as being problematic by one of skill in the art.

Furthermore, , in general, antibody expression was not a predictable art at the time the invention was filed, and a particular host cell, vector system or expression strategy could not have been used with a reasonable expectation of success. One of skill in the art would recognize the validity of these assertions because several research papers point out that antibodies are difficult to express in certain systems. Samples from a small number of scientific publications are provided herewith, and discussed below:

Ward et al (Protein Engineering 6: 981-1988, 1993), showed several problems were associated with the expression of a single antibody, e.g. plasmid loss, poor secretion of the antibody, unstable light immunoglobulin chains and cell lysis (see the section entitled "Expression", starting in the second

column of page 984). These problems appear to be overcome by switching vector systems, and using a completely different promoter to express the light chain (see the first sentence of the section entitled "Stabilization studies" in the first paragraph of page 986).

Kreissig et al (Chapter 3 of Immunoanalysis of Agrochemicals: Sequence Analysis of Individual Chains of Antibodies, 1995) states that "It is also thought to be very difficult to produce a functional whole antibody (Figure 1) in prokaryotic hosts, presumably because of the large size of the antibody, in addition to the absence of cellular machinery necessary for complex glycosylation of recombinant proteins produced in prokaryotes", (see page 39) indicating that glycosylation is important for antibody function, and that it is not clear which cells provide an adequate glycosylation for antibody function.

Ward et al (Chapter 15 of New Frontiers in Agrochemical Immunoassay: Development and Application of Recombinant Antibodies to Pesticide Residue Analysis, AOAC International Press), states that "for successful production of functional Fabs, it may be desirable to use promoters that are not as strong as the one described above, or to exert very tight control over the expression such that sufficient growth of the bacterium could be ensured prior to induction of antibody production", (see page 207) indicating that the choice of promoter is very important to facilitate antibody production. The reference further states that "the presence of different leader sequences preceding the heavy and light-chain coding sequences might interfere with proper assembly of the recombinant chains into functional Fab/F(ab')$_2$", (see page 209) indicating that the choice of leader sequence is very important for antibody expression. The reference further states that "The use of a very strong and leaky promoter system, in our experience, was detrimental to cell growth and was not sustainable. This led us to believe that tightly regulated promoters of moderate strength such as housekeeping gene promoters bay be better for antibody production. We have found that, as reported for other proteins, the choice of host *E.coli* strains is also important" (see page 210). This reference again indicates that choice of promoter is very important and teaches that , even within strains of a single species, there is variability in antibody expression.

E. coli

In view of the technical difficulties associated with constructing a dual expression cassette vector in which the expression cassettes contain related nucleic acid sequences, and the problems associated with antibody expression in general, one of skill in the art would not make and use the claimed vector with a reasonable expectation of success. As such, any rejections directed to such vectors may be withdrawn.

*Summary*

The Office Action points to several references that assertedly provide all the elements of the claimed invention. However, Robinson, cited for disclosing a key claim element -- a dual expression cassette vector for antibody production -- when read as a whole, undeniably leads one of skill in the art *away* from using a dual expression cassette vector for antibody expression in yeast. As such, Robinson provides no motivation to one of skill in the art to express an antibody in *Pichia* using a dual expression cassette vector. This deficiency is not met by any of the other references cited by the Office, and, as such, one of skill in the art would not be motivated to combine the disclosures cited by the Office in making this rejection, especially in light of the Robinson disclosure.

Also, applicants have provided further evidence that shows that antibody production is not straightforward and, at the time of filing, would not have been practiced with a dual expression cassette vector in Pichia with any reasonable expectation of success. Thus, claims 36-40 and 42-49 are not obvious under 35 U.S.C. §103(a) over Horwitz in view the secondary references, and, accordingly, this rejection may be withdrawn.

With respect to claim 41, Vanderlaan, cited for disclosing an anti-dioxin antibody, also fails to provide motivation to express an antibody in *Pichia* using a dual expression cassette vector, and accordingly, provides no motivation to combine the cited references. As such, claim 41 is not obvious under 35 U.S.C. §103(a) over Horwitz in view the secondary references and Vandarlaan, and this rejection may be withdrawn.

## III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number UCAL-269.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 11-18-2002

By: James Keddie
James S. Keddie Ph.D.
Registration No. 48,920

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

F:\DOCUMENT\UCAL\269\Response to oa dated 7-18-2002.doc